

# Dendrimer–Silver Complexes and Nanocomposites as Antimicrobial Agents

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## ABSTRACT

Silver complexes of poly(amidoamine) (PAMAM) dendrimers as well as different {silver–PAMAM} dendrimer nanocomposite solutions have been tested in vitro against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria, using the standard agar overlay method. Both PAMAM silver salts and nanocomposites displayed considerable antimicrobial activity without the loss of solubility and activity, even in the presence of sulfate or chloride ions.

**Introduction.** The usefulness of silver as an antimicrobial agent has been known for a long time. It is an effective agent with low toxicity, which is especially important in the topical antibacterial treatment of burn wounds, where transient bacteremia is commonly cited.<sup>1</sup> Silver sulfonamides, particularly silver sulfadiazine<sup>2</sup> (AgSD), have been used as the standard treatment for burns over the past two decades, either alone or in combination with other antibiotics,<sup>3</sup> cerium,<sup>4</sup> and zinc compounds.<sup>5</sup> It was suggested that the basic function of the almost insoluble AgSD may be the slow release of silver into the superficial wound environment.<sup>6</sup> Silver chloride was assumed to form at the burn wound, and the absorption of silver was believed to be negligible.<sup>7</sup> In accordance with this assumption, it was found that serum and tissue silver levels with 10% silver nitrate used in burns produced no difference from that of 1% silver sulfadiazine cream.<sup>8</sup> However, sulfonamide-resistant organisms have been reported as a frequent consequence of the clinical use of sulfadiazine silver.<sup>9</sup> It was also shown that the sulfadiazine component is not necessary for in vitro sensitivity, and the use of sulfadiazine silver in every case can, therefore, lead to the selection of organisms that are resistant not only to sulfonamides but also to antibiotics of clinical consequence.<sup>10</sup> In a new approach, silver nylon dressings were tested on *Pseudomonas aeruginosa*-infected burn wounds. Silver nylon

was therapeutic when used as a surface anode or cathode, and, to some extent, even without any applied weak direct current. This result indicated that silver nylon dressings may be a valuable antimicrobial burn wound covering device.<sup>11</sup> Despite major advances in burn wound management and other supportive care regimens, infection remains the leading cause of morbidity in the thermally injured patient,<sup>12</sup> and the search for different treatments and new ideas is continuing.

The application of silver-binding membranes has recently been suggested to further reduce the likelihood of silver toxicity to retard the movement of silver ions and minimize silver absorption at a healing wound.<sup>13,14</sup>

Dendrimers<sup>15–19</sup> are symmetrical and spherical macromolecules, comprising a relatively dense shell composed of a core, branching sites, and terminal groups that usually form a well-defined surface. Their interior may be similar or very different from the surface of the molecule. Chemical and/or physical properties, such as reactivity, complex or salt formation, hydrophilicity, and so forth can be varied and optimized. Poly(amidoamine) PAMAM dendrimers are obtained by the iterative branching of  $\beta$ -alanine repeat units. Due to their biofriendly nature<sup>20–22</sup> and unique carrier properties, they show promise to outperform other polymeric materials<sup>14</sup> for medical applications. Diffusion of dendrimers through membranes is the function of generation (due to their spherical and monomodal character), and appropriately selected membranes may retain dendrimer hosts with 100% selectivity. PAMAMs are also stable and soluble in water.

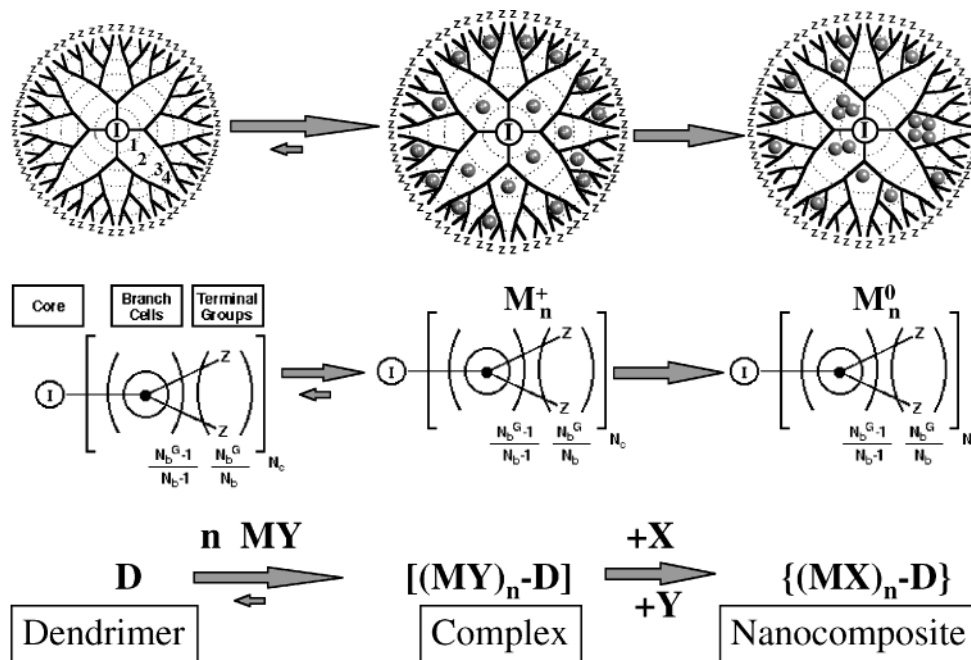
Dendrimers are now recognized as monodispersed nano-reactors possessing architectures and ligand sites that allow

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**Figure 1.** General formation scheme of PAMAM dendrimer complexes and nanocomposites. PAMAM structural subunits: core = ethylenediamine, branching site =  $-\text{N}<$ , chains connecting the branching sites =  $-\text{CH}_2\text{CH}_2\text{CONHCH}_2\text{CH}_2-$ . Terminal groups on the surface are marked as  $-\text{CH}_2\text{CH}_2-\text{CO}-\text{Z}$ . For the investigated dendrimers (TRIS and carboxyl surfaces),  $\text{Z} = -\text{NHC}(\text{CH}_2\text{OH})_3$  and  $\text{Z} = -\text{OH}$ , respectively.

the pre-organization of metal ions<sup>23,24</sup> on their surface and/or in their interior. Unique physical and chemical properties have been observed in dendrimer-based complexes and nanocomposites<sup>25–28</sup> as a consequence of the atomic/molecular level dispersion of the guest in a dendrimer host.

As a large number of polar terminal groups are concentrated within a relatively thin shell of the macromolecules, an extremely high local concentration of silver may be provided by the cations of dendrimer carboxylate salts. Simultaneously, the accessible tertiary nitrogens in the interior may give rise stable complexes with ions of transition metals, including silver. PAMAMs are able to solubilize many organic materials that are commonly considered insoluble in water through guest–host interactions. Bioactive materials, including metals, metal ions, and organic molecules, therefore, may be combined in variable concentrations and composition in one nanoscopic delivery vehicle when PAMAM dendrimers are used. Studies using antibody/dendrimer conjugates in vitro and in vivo in experimental animals have documented these conjugates to be nontoxic and able to target biologic agents to specific cells.<sup>21,22,29</sup>

In this work, surface-modified poly(amidoamine) (PAMAM) dendrimers were utilized as templates/nanoreactors/containers to pre-organize silver ions and subsequently contain them in the form of solubilized and stable, high surface area silver domains (Figure 1).

**Experimental Section. Materials.** Amine terminated PAMAM dendrimers were purchased from Dendritech and were used without further purification. All other reagents were purchased from the Aldrich Chemical Co. and were used as received.

**Instrumentation.** IR spectra were recorded on a Nicolet 20DBX FT-IR spectrophotometer between  $\text{CaF}_2$  plates, UV–

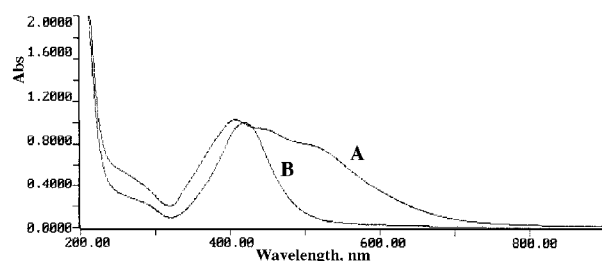
vis spectra were obtained on a Cary 1E spectrophotometer at room temperature between 200 and 900 nm in a Suprasil 300 quartz cell ( $L = 1$  mm).  $^1\text{H}$  and  $^{13}\text{C}$  NMR measurements were carried out by a Varian Unity 300 multinuclear spectrometer equipped with a temperature controller. Size Exclusion Chromatography was performed on three TSK gel columns (4000, 3000, and 2000) using a Waters 510 pump with a Wyatt Technology Dawn DSP–F MALLS, Wyatt Technology 903 interferometric refractometer, and a Waters 410 differential refractometer, respectively. A Phillips EM301 instrument was applied for transmission electron microscopy (TEM) using Formvar coated carbon grids. Atomic absorption measurements were done at the Independent Testing Laboratory of the Saginaw Valley University, MI. Image analysis was performed on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>).

**Sample Preparation.** PAMAM\_E4.NHC( $\text{CH}_2\text{OH}$ )<sub>3</sub> a generation four, ethylenediamine (EDA) core, dendrimer bearing tris(2-hydroxymethyl)-amidomethane (TRIS) termini with aliphatic OH-surface (for short: E4.T), and generation five, EDA core PAMAM dendrimer with carboxylate terminal groups (E5.OH) were used. Silver containing PAMAM complexes were prepared simply by adding aqueous solutions of the dendrimers to the calculated amount of silver acetate powder. Although  $\text{CH}_3\text{COOAg}$  is hardly soluble in water, it quickly dissolves in the PAMAM solutions. This enhancement is due to the combined action of the silver-carboxylate salt formation and/or to the complex formation with the internal nitrogens. This procedure results in slightly yellow dendrimer–complex/salt solutions, that very slowly photolyze, when exposed to light, into dark brown, metallic silver-

**Table 1:** Effectivity of PAMAM-silver Complexes and Nanocomposites<sup>a</sup>

tested compound	% dendrimer	% Ag(0)	<i>S. aureus</i>	<i>Ps. Aeruginosa</i>	<i>E. coli</i>
{Ag(0)-E5.OH}	2.29	0.45	11.0	10.2	8.6
{AgCl-E5.OH }	2.82	0.45	10.0	9.5	8.8
[AgAc-E5.OH]	3.66	0.81	12.0	10.5	8.7
[AgNO <sub>3</sub> -E5.OH]	1.76	0.31	10.3	9.7	9.0
[AgAc-E4.T]	3.66	0.66	10.9	10.4	9.5
[AgNO <sub>3</sub> -E4.T]	1.76	0.35	11.1	10.2	9.4
[AgAc-E4.T]	9.06	0.82	11.8	10.3	9.3
[AgAc-E5.OH]	10.0	2.54	14.7	12.85	10.15
E5.ONa	3.66		6	6	6
E4.T	3.72		6	6	6
10% AgNO <sub>3</sub>		6.35	12.85	11.05	9.8
7.5% AgNO <sub>3</sub>		4.76	12.7	11.1	9.65
5% AgNO <sub>3</sub>		3.17	12.65	11.2	9.5
2.5% AgNO <sub>3</sub>		1.58	12.55	11.2	9.7
1% AgNO <sub>3</sub>		0.635	12.3	11.1	9.8
0.5% AgNO <sub>3</sub>		0.317	12.05	10.7	9.9

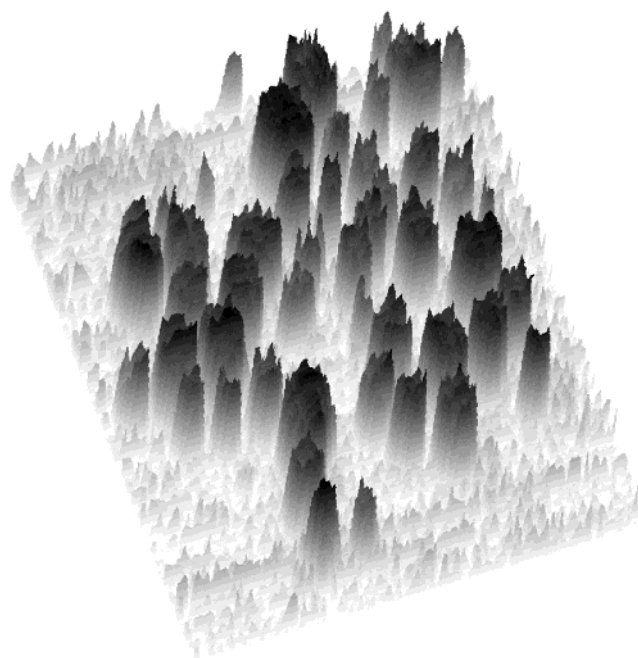
<sup>a</sup> Sensitivity data are presented as the diameter of the inhibited area in mm. Standard disks of 6 mm diameter were used therefore a value of 6 represents no inhibition of growth beyond the edge of the disk.



**Figure 2.** Comparison of UV-vis spectra of the two nanocomposites, A = {(Ag(0))<sub>256</sub>-PAMAM\_E5.OH} and B = {(Ag(0))<sub>10</sub>-PAMAM\_E4.T}.

containing dendrimer-silver nanocomposite solutions. Final concentrations of the samples were confirmed by atomic absorption spectroscopy. Detailed structural characterization of similar silver nanocomposites can be found in the literature.<sup>27</sup>

**Antimicrobial Testing.** The standard agar overlay method was used. In this test, dendrimer-silver compounds were examined for diffusible antimicrobial activity by placing a 10  $\mu$ L sample of each solution onto a 6 mm filter paper disk and applying the disk to a dilute population of test organisms distributed over an agar growth medium. Two parallel tests were run, and 10  $\mu$ L of each agent was used per disk. The standard 24 antibiotic disk panel was run each time as a control. Test organisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) were also run against a series of dilution of silver nitrate, ranging from 10% to 0.5%. **Results and Discussion.** Comparison of the UV-vis spectra of these nanocomposite solutions (Figure 2) clearly demonstrates the formation of the uniform silver domains when E4.T is used, versus the dissimilar silver clusters (Figure 3) formed in the dendrimer carboxylate solution. As the PAMAM host is an aliphatic compound, it does not have visible absorption over 300 nm wavelength. Absorption at longer wavelength is caused by the silver nanoparticles. Spec-



**Figure 3.** Surface plot of a selected area of a TEM image acquired from the aqueous solution of the {(Ag(0))<sub>256</sub>-PAMAM\_E5.OH} nanocomposite. The width of objects is proportional to the size of the clusters, whereas height/darkness is proportional to the relative amount of silver in the individual nanocomposite particles.

trum A of {(Ag(0))<sub>256</sub>-PAMAM\_E5.OH} is a typical external nanocomposite spectrum: an envelope of three peaks due to a mixture of three different but well-defined silver domain sizes. Spectrum B of {(Ag(0))<sub>10</sub>-PAMAM\_E4.T} is a typical internal nanocomposite spectrum displaying a single and symmetrical spectral peak for silver nanocomposite particles that are smaller but they have a uniform distribution.

Both the silver-dendrimer complexes and the nanocomposites displayed antimicrobial activity comparable or better to those of silver nitrate solutions. All silver nitrate solutions displayed approximately the same activity probably due to the same limited solubility of the secondary silver compounds that were formed in the test media. Interestingly, increased antimicrobial activity was observed with dendrimer carboxylate salts. We have attributed this effect to the very high local concentration (256 carboxylate groups around a 54 Å diameter sphere)<sup>30</sup> of nanoscopic size silver composite particles that are accessible for microorganisms. These silver domains—either in the form of Ag<sup>+</sup>, Ag(0), or any other compound, such as AgCl or Ag<sub>2</sub>SO<sub>4</sub>—are bound to the dendrimer with a calculated specific surface area of several thousand m<sup>2</sup>/g. The antimicrobial activity was smaller when internal silver complexes were applied, indicating, that the accessibility of the silver is an important factor.

Due to the very small size of the dendrimer templates used (45–54 Å), these silver compounds photolyze very slowly. Silver compounds, reduced into Ag(0) in the absence of dendrimers, quickly formed micron-size crystallites with little adherence to surfaces. Reduction of silver-dendrimer complexes or salts resulted in nanometer sized clusters that adhered strongly to quartz and glass, similarly to gold

dendrimer nanocomposites.<sup>31</sup> In dialysis experiments, silver was retained in the dendrimers, and the guest and host virtually diffused together as if they were simple macromolecules. In the absence of dendrimers, silver ions quickly precipitated in the form of insoluble silver salts when contacted with chloride and sulfate ion containing solutions. When conjugated to a dendrimer, the silver ions were transformed into stable silver nanocomposites that remained soluble in the solvent.

**Conclusions.** Poly(amidoamine) dendrimer based silver complexes and nanocomposites proved to be effective antimicrobial agents in vitro. Due to the atomic/molecular level dispersion of the guest in a dendrimer host, the activity is retained if the microorganism is able to contact with the organized silver domains of the nanocontainers. Macroscopically, the silver remained conjugated to the dendrimer in the form of ions, stable metallic silver clusters or silver compounds. Because the dendrimer host is soluble, it is able to deliver the immobilized silver in the agar medium by its own diffusion. The silver clusters remain active because of their extremely high surface area. Reaction with chloride and sulfate ions neither blocks the diffusion of the silver nor the activity against *S. aureus*, *Ps. aeruginosa* and *E. coli*. The protected silver and silver compounds displayed high antimicrobial activity in several cases without the loss of solubility. However, the diffusion of dendrimers can be totally prevented if common cellulose membranes are used.

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